Ameliorative Effect of *Momordica Charantia* against Arsenic Induced Hepatotoxicity in Albino Mice

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In our present study, an attempt has been made to study the effect of Arsenic trioxide on biochemical parameters and ameliorating effects of the aqueous fruit (vegetable) extract of *Momordica charantia* L (MC), Karela, (Bitter Melon) in swiss albino mice. The aim and purpose of our study was to evaluate the ability of aqueous extracts from the whole fruit *Momordica charantia* L to protect normal hepatocytes against oxidative damage *in-vitro*. The present study was conducted to evaluate the protective role of *Momordica* against arsenic induced hepatotoxicity in albino mice. Albino mice were divided into three groups. Our results imply that *Momordica charantia* L has the protective antioxidant properties. Group I were control mice, Group II received an acute dose of arsenic (5 mg/kg bw) orally, Group III received an acute dose of arsenic followed by daily administration of *Momordica* (20mg/kg bw) orally. Autopsies were done on 15 days post treatment. Arsenic trioxide treatment leads to increase in weight of liver. Biochemical analysis of treated group showed decrease in antioxidant enzymes, viz., SOD, CAT but increased MDA content in liver as compared to control group. MC administration to mice decreased the weight of mice and showed significant protection in the alleviation of arsenic induced hepatic injury.

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1. Introduction

Increasing evidence suggests that oxidative damage to cell components may play an important pathophysiological role in many types of human diseases. Several reports have shown that erythrocyte attack of reactive oxygen species (ROS) is the key event in β-thalassemia, sickle cell anemia, glucose-6-phosphate dehydrogenase deficiency, and other hemoglobinopathies. Erythrocytes, potentially powerful promoters of oxidative processes, are extremely susceptible to oxidative damage as a result of the high polyunsaturated fatty acid (PUFA) content of their membranes and the high cellular oxygen and hemoglobin (Hb) concentrations.

Malondialdehyde (MDA), the well characterized product of the lipid peroxidation of erythrocytes, is a highly reactive and bifunctional molecule, which has been shown to cross-link erythrocyte phospholipids and proteins to impair a variety of the membrane-related functions, which ultimately lead to diminished erythrocyte survival (hemolysis). Further, erythrocyte lipid peroxidation may be involved in normal cell aging, and it has been associated with a variety of pathological events. Oxidants also produce alterations in erythrocyte membranes as
manifested by a decreased cytoskeletal protein content (low-molecular-weight, HMW), and production of high molecular weight (HMW) proteins\textsuperscript{12,13}, which can lead to abnormalities in erythrocyte shape and disturbances in the microcirculation\textsuperscript{14}.

*Momordica charantia* L (Bitter melon), Karela, is one of the most important species belonging to the family Cucurbitaceae, commonly known as bitter gourd or bitter melon in English. The origin of this crop is presumed to be India, with secondary center of diversity in China. Its fruits, leaves and roots have been shown to exhibit various biological activities, including antidiabetic, anti-inflammatory, antiulcer, anti-infectious and antitumor, and is used for treating jaundice, leprosy and as antivenom to snakebite\textsuperscript{15,16}. A bitter melon fruit has a particular clinical usefulness, similar to MAP30 (*Momordica* anti-HIV protein, weight: 30 kDa) that is believed to have multiple functions that could be beneficial for the treatment of HIV infections. Recently, it has been found to be a powerful activator of peroxisome proliferator-activated receptor that regulates the expression of genes involved in lipid metabolism and transport\textsuperscript{17}. In addition, examination of the phytochemicals of this plant indicated the presence of active components like momorcharins, momordenol, momordicinil, momordicinis, momordicin, momordin, momordolol, charantin, charine, cryptoxanthin, cucurbitins, cucurbitacins, cucurbitanes, cycloartenols, diosgenin, elaostearic acids, erythroidol, galacturonic acids, gentisic acid, glyaglycosides, goyasaponins and multiflorenol, which have been isolated\textsuperscript{18,19}. It is a well-documented fact that most medicinal plants are enriched with phenolic compounds and bioflavonoids that have excellent antioxidant property\textsuperscript{20}. Although various phytochemical constituents and diverse medicinal activities have been attributed to this plant, no biochemical studies have been carried out to shed light on the role of *M. charantia* fruit extract on haemotological and marker enzymes, lipid peroxidation and antioxidant status in experimental arsenic induced toxicity.

2. Materials and Methods

2.1 Chemicals

All the fine chemicals were procured from Sigma Chemicals & Co., Ltd., St.Louis, MO, USA. All other chemicals used were of good quality and analytical grade.

2.1.2 Oxidative Stress

As remarkable as our antioxidant defense system is, it may not always be adequate. The term “oxidative stress” has been coined to represent a shift towards the pro-oxidants in the pro-oxidant/antioxidant balance that can occur as a result of an increase in oxidative metabolism. Increased oxidative stress at the cellular level can come about as a consequence of many factors, including exposure to alcohol, medications, trauma, cold, infections, poor diet, toxins, radiation, or strenuous physical activity. Oxidative damage to DNA, proteins, and other macromolecules has been implicated in the pathogenesis of a wide variety of diseases, most notably heart disease and cancer\textsuperscript{21}. Growing bodies of animal and epidemiological studies as well as clinical intervention trials suggest that antioxidants may play a pivotal role in preventing or slowing the progression of both heart disease and some forms of cancer\textsuperscript{22,23}.

2.1.3 Sample Preparation

Fresh Fruits of *Momordica charantia* L (MC), Karela, were collected from the local market. One kilogram of unripe fruits was thoroughly washed, and the seeds were removed. The aqueous extract of the fruit was prepared and was administered orally to the mice.

2.1.4 Animal Model

Male albino mice (20±2g), (*Rattus novergicus*) were procured from Tamil Nadu University for Veterinary and Animal Sciences, (TANUVAS) Chennai, India were used for the study. Animals were fed with commercially available standard rat pelleted feed (M/s Pranav Agro Industries Ltd., India) under the trade name Amrut rat/mice feed and water was provided *ad libitum*. The rats were housed under conditions of controlled temperature (22±2°C) and acclimatized to 12-h light, 12-h dark cycle. Animal experiments were conducted according to the guidelines of institutional animal ethical committee. All the drugs (standard and test as well as vehicle were administered per-orally using insulin syringe.

3. Experimental Design/Segregation of Groups

Experimental animals were divided into three groups of three mice each as follows. Arsenic was dissolved in double distilled water and administered orally to mice.

Group I : Served as Control mice and were given distilled water.

Group II : Served as arsenic induced, mice received 5mg/kg bw of Arsenic orally for 15 days.

Group III : Animals were injected with an acute dose of 5 mg/kg bw of Arsenic followed by a daily dose of 20 mg/kg bw of *Momordica* for 15 days through Gavage’s method.

3.1 Animal Sacrifice and Sample Collection

After the last dose, animals were fasted for 12 hours and sacrificed. Blood samples were collected by orbital sinus puncture, Herck method. Serum was prepared following procedure. Briefly, blood samples were withdrawn from orbital sinus using non heparinised capillary tubes, collected in dried centrifuge tubes and allowed to clot. Serum was separated from the clot and centrifuged at 3000
rpm for 15 min at room temperature. The serum was collected carefully and kept at 20°C until analysis. Glucose, Serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) activities were measured according to the method described by King et al., 1965a, while bilirubin activity was measured.

3.2 Statistical Analysis

The data were analyzed by using Student’s t-test.

4. Results and Discussions

Arsenic is highly toxic and corrosive to gastrointestinal tract resulting into partial anorexia and gastrointestinal disturbances followed by loss of body weight in arsenic intoxicated animals as compared to control animals evaluated that arsenic at high doses causes acute hepatic injury and hepatocellular necrosis causing leakage of hepatocellular enzymes into blood further, they observed that the extent of injury to the hepatocytes is generally detected by the activity of antioxidant enzymes. In the present study, it is observed that the relative weight of liver was more in arsenic exposed mice in comparison to control mice. This observation is in confirmation with the study, they also reported that liver weight significantly increased in male rats fed with 100 mg/kg arsenic diet for a period of 2 weeks. As liver is a very active site of metabolism, it is the main site of arsenic intoxication, where the arsenic methyltransferase enzymes mediate the methylation process with S-adenosylmethionine as the methyl donor and GSH as an essential co-factor. The measurement of lipid peroxidation byproducts and the status of antioxidant enzymes like SOD and CAT are appropriate indirect ways to assess the prooxidant-antioxidant status in the tissues and the estimation of MDA, a by-product of lipid peroxidation, continues to be a reliable method to assess the degree of peroxidative damage to cell membrane. In the present investigation, lipid peroxidation was enhanced, while the activities of SOD and CAT were significantly decreased in the liver of treated mice. The liver can accumulate arsenic with repeated exposures. Liver is the main site of synthesis of proteins, therefore, hepatic damage caused by arsenic leads to decrease in the levels of antioxidant enzymes with arsenic trioxide. Recent studies have clearly demonstrated that arsenic compounds during their metabolism in cells generate reactive oxygen species like superoxide anion, hydroxyl radical and hydrogen peroxide leading to oxidative stress suggested. Enhanced production of free radicals and inhibition of antioxidant enzymes as possible mechanisms to explain arsenic induced oxidative damage. A significant reduction in SOD levels was observed in both the experimental groups (group II and III) as compared to control group suggested that superoxide dismutase is an important antioxidant enzyme responsible for the elimination of superoxide radical. The exhausted SOD levels observed in this study might be due to overproduction of free radicals in the body. A decrease in the activity of SOD can be owed to an enhanced superoxide production during arsenic metabolism. SOD catalyzes the dismutation of superoxide anions and prevents the subsequent formation of hydroxyl radicals. In the present study, the decreased SOD activity in liver of mice suggested that the accumulation of superoxide anion radical might be responsible for increased lipid peroxidation following arsenic treatment. In the present study a decrease in catalase activity in liver, reported that superoxide radical also inhibited the activity of catalase. Exposure to arsenic decreased the catalase activity, also observed that Arsenic inhibited the catalase activity in human fibroblast cells. CAT catalyzes the removal of H$_2$O$_2$ formed during the reaction catalyzed by SOD. So, the present study, the decreased CAT activity indicated that exposure to arsenic may result in impaired ability to detoxify H$_2$O$_2$ via catalase and accumulation of H$_2$O$_2$ occurred in liver of mice. A significant increase in lipid peroxidation in arsenic treated group as compared to control. It is well known that tissue retention of arsenic increases with the length of exposure due to the lesser rate of excretion of arsenic than exposure which causes its accumulation in tissues. Liver being the main workhouse of metabolism, arsenic was accumulated at high concentration in liver of the arsenic exposed groups in the present study, demonstrated a tendency for a positive correlation between arsenic concentration and lipid peroxidation level in liver, kidney and heart of rats following acute exposure to arsenic, reported that GaAs induced lipid peroxidation in blood, liver and kidney of rats. Their study indicated that the lipid peroxidation was increased by high arsenic level and duration of exposure. Arsenic induced MDA production could be due to the impairment of cells natural protective system. In this study, there was an inhibition of peroxidative damage as evidenced by reduced MDA level, and elevation of CAT and SOD activities in the arsenic and Momordica co-treated mice. This finding is consistent with previous findings that Momordica significantly lowered lipid peroxidation by maintaining the of the antioxidant enzymes; SOD, CAT and GSH in the rat testes activities. Recently, much attention has been focused on the protective effects of antioxidants and the possibility of using antioxidants in the treatment of hepatocytic toxicity. Accumulating evidences suggest that the effects of Momordica against oxidative damage may be attributed to its antioxidant properties. The prevention of arsenic trioxide induced oxidative stress in mice by Momordica charantia L, shown by the results obtained from this study, suggests the protective effects of Momordica against arsenic trioxide induced toxicity. Thus, Momordica supplementation aids in amelioration of hepatotoxicity induced by arsenic trioxide in albino mice.
Fig. 1. Body weight of mice in control and treated groups.

Fig. 2. Weight of liver in control and treated groups.

Fig. 3. SOD activity in liver of control and treated group.

Fig. 4. CAT activity in liver of control and treated group.

Fig. 5. MDA activity in liver of control and treated group.
5. Summary & Conclusion

Our study demonstrates that *Momordica charantia* L is a rich source of potential antioxidants and such effects may be related to their biochemical. The study was carried out to investigate the hepatoprotective effect of aqueous extract of *Momordica charantia* L on Arsenic trioxide induced hepatic cellular damage on male wistar rats. The *Momordica charantia* L extract showed Serum levels of Superoxide dismutase (SOD) and Catalase (CAT) were significantly (P<0.05) lower in the animals that received extract than control. However, Malondialdehyde (MDA) levels were significantly elevated (P<0.05) in the rats that received the extract of *Momordica charantia* L when compared with the controls. The *Momordica charantia* L extract displayed antioxidant effect. Histopathological studies revealed alterations of the hepatic tissue caused by Arsenic trioxide exposure were prevented by *Momordica charantia* L extract administration. Biochemical analysis of treated group showed decrease in antioxidant enzymes viz., SOD, CAT but increased MDA content in liver as compared to control group. MC administration to mice decreased the weight of mice and showed significant protection in the alleviation of arsenic induced hepatic injury.

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